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REACTIONS OF N-ETHYL- (HN-1), N-METHYL-BIS(2-CHLOROETHYL)AMINE (HN-2), AND TRIS(2-CHLOROETHYL)AMINE (HN-3) WITH PEROXIDES

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The chemical decontamination of nitrogen mustards, HN-1, -2, and -3, generally involves oxidation and hydrolysis; bleach is the most commonly used reagent. Reactions of nitrogen mustards with peracetic acid and 30% hydrogen peroxide were studied, and the products were analyzed by proton and ^{13}C NMR spectra.

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PREFACE

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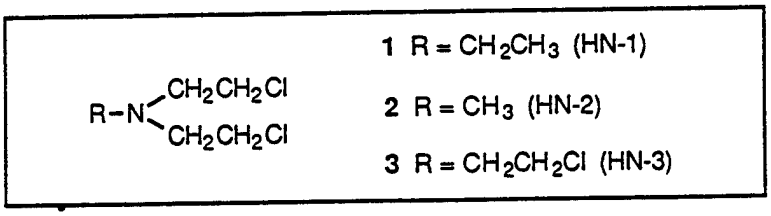
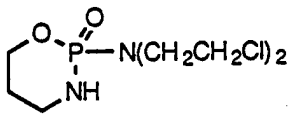
REACTIONS OF N-ETHYL-(HN-1), N-METHYL-BIS(2-CHLOROETHYL)AMINE (HN-2), AND TRIS(2-CHLOROETHYL)AMINE (HN-3) WITH PEROXIDES

1. INTRODUCTION

Ethyl- (HN-1) (1), methylbis(2-chloroethyl)amine (HN-2) (2) and tris(2-chloroethyl)amine (HN-3) (3) are nitrogen mustards used as chemical warfare agents.[1] Nitrogen mustards are chemically reactive species where the chloride moiety reacts readily with nucleophilic centers. Alkylation of biological active components involves attack at the nitrogen, sulfur or oxygen atoms of the living cell building blocks, amino acids and nucleic acids. This nucleophilic reaction mechanism accounts for their biological effects which include blister formation and anticancer activity. Structural modification of the nitrogen mustards to alter the chemical and physical properties has led to the development of useful antineoplastic agents, such as cyclophosphamide (4) and uracil mustard (5).[2] The N-oxides of the nitrogen mustards have also been shown to be more effective and less toxic than their corresponding tertiary amines in treating Yoshida sarcoma in rats.[3]





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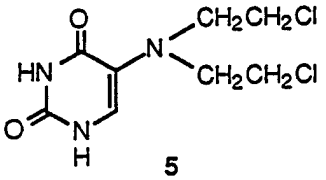

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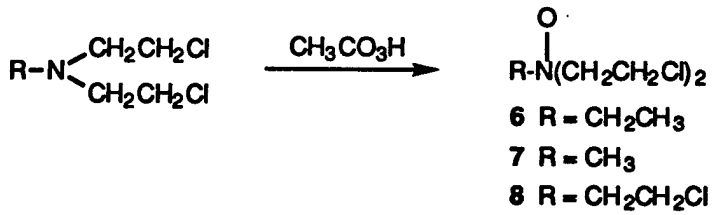
Chemical decontamination of the poisonous nitrogen mustards generally involves oxidation and hydrolysis; bleach is the most commonly used reagent.[4] These treatments presumably convert these mustards into their corresponding N-oxide and the hydrolyzed products. In this study, we are interested in the synthesis of the N-oxide of nitrogen mustards and also the reaction of HN-1, HN-2 and HN-3 with hydrogen peroxide which is one of the oxidizing agents commonly used in the decontamination of chemical warfare agents.

2. CHEMISTRY

The preparation of the N-oxide of HN-1, HN-2 and HN-3 from the reaction of nitrogen mustards with peracetic acid, prepared from perborate and acetic anhydride, was reported by Bergmann and Stahmann in 1946.[5] In one of our projects, we required HN-3 N-oxide (8) as a reference sample, thus, a slight modification of literature procedure was developed to prepare 8. Reaction of HN-3 and commercially available peracetic acid (32% solution in acetic acid) under slightly basic conditions, followed by treatment with hydrochloric acid afforded the corresponding N-oxide hydrochloride salt 8 as a white solid identical to the literature compound (Scheme 1).

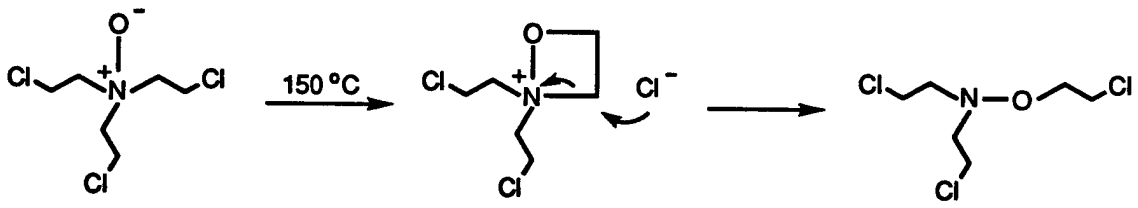
Scheme 1



6 R = CH_2CH_3
7 R = CH_3
8 R = CH_2CH_2Cl

Amine N-oxides are useful intermediates for the synthesis of other organic compounds. The thermolysis of amine N-oxides produces olefins *via* a five-membered ring β-elimination Cope reaction mechanism.[6] The presence of chlorine as the leaving group in nitrogen mustard N-oxides alters the reaction mechanism. Thus, nucleophilic substitution instead of elimination becomes the major course of this thermal reaction. When **8** hydrochloride was either heated in an oil bath at 150 °C under nitrogen or heated with powdered potassium carbonate (used as the base) at this temperature, it rapidly transformed into N-(2-chloroethoxy)-N,N-bis(2-chloroethyl)amine (**9**), presumably *via* the mechanism shown in Scheme 2. Identical but slower rearrangement of **8** hydrochloride also occurred in aqueous solution at ambient temperature indicating the unstable nature of the nitrogen mustard N-oxides. Similar results have also been reported by Szafraniec *et al.*[7] in the reaction of HN-1 and *m*-chloroperbenzoic acid in chloroform in which partial rearranged product was also observed by NMR analysis of the reaction mixture.

Scheme 2



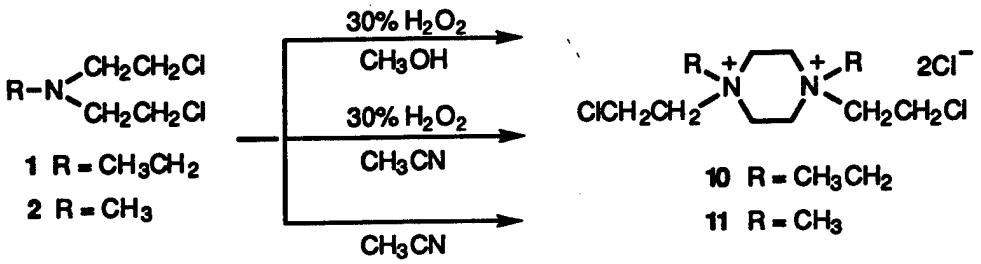
8 **9**

Hydrogen peroxide is one of oxidizing reagents commonly used in the oxidation of a tertiary amine to its N-oxide.[6] Accordingly, a homogeneous solution of HN-1 (**1**) and 30% hydrogen peroxide in methanol or acetonitrile at room temperature produced white precipitate in less than an hour, with a more profound effect in methanol. The white solid was soluble in water, aqueous acidic or basic solution indicating a charged species might be formed. After an extremely careful examination of the ^1H and ^{13}C NMR spectra and comparison with known piperazine compounds, the chemical structure of the white solid was identified as the bispiperazine quaternary ammonium salt **10** as shown in Scheme 3. Under identical reaction conditions, the bis-quaternary ammonium salt **11** was produced from HN-2 (**2**) in the same manner. It was surprising that HN-1 and HN-2 were not oxidized by 30% hydrogen peroxide under these conditions. The cyclized dimeric bisquaternary ammonium salts **10** and **11** were also formed rapidly in acetonitrile alone at ambient temperature. These results suggest that HN-1 and HN-2 proceed *via* a bimolecular nucleophilic substitution at a rapid rate in polar solvents which lead to the formation of bis-quaternary ammonium salts and thus, completely blocks the oxidation reaction at the nitrogen.

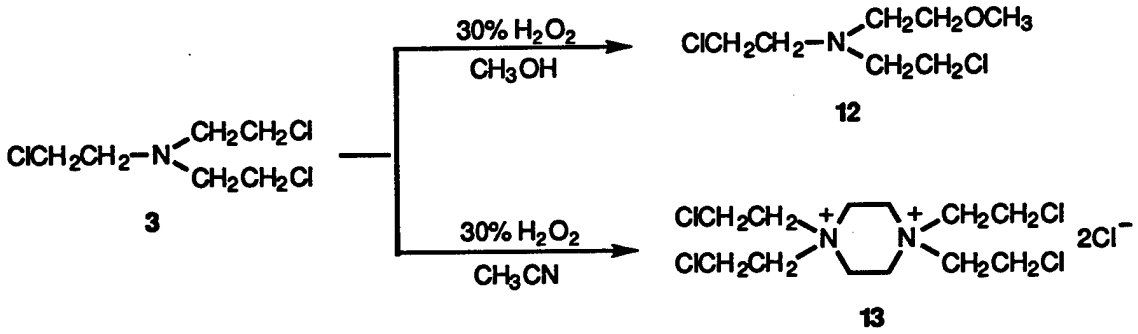
Under the same conditions, a mixture of HN-3 (**3**) and 30% hydrogen peroxide in methanol at room temperature yielded a new compound **12** in which a methanol molecule was incorporated into the product. When acetonitrile, a weaker nucleophile, was used to replace methanol as the solvent, a bi-molecular nucleophilic substitution took place as in the case of HN-1 and HN-2, but at a much slower rate. The cyclized bis-quaternary ammonium salt **13** was isolated in only three percent yield, and the remaining was

the starting material, HN-3 (Scheme 4). This slow reaction was also observed in the acetonitrile-only media. The solution of HN-3 in acetonitrile did not form any cyclized quaternary salt after 14 days. Addition of a small amount of water into the mixture did enhance the formation of **13**, but in very low yield. These results suggest that HN-3 is less reactive than HN-1 and HN-2 and water is the favored solvent for formation of bisquaternary salts.

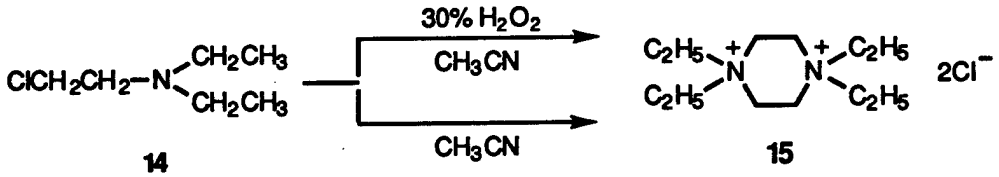
Scheme 3



Scheme 4



Scheme 5



With these interesting results, we decided to include 2-(diethylamino)ethyl chloride (**14**), with one 2-chloroethylamino functionality in the molecule, into our studies. A homogeneous solution of **14** and 30% hydrogen peroxide in acetonitrile produced N,N,N',N'-tetraethylpiperazinium dichloride (**15**) as a white precipitate in quantitative yield indicating that a facile bi-molecular reaction also occurred to a molecule containing single 2-chloroethylamino group as shown in Scheme 5. Similar to HN-1 and HN-2, the acetonitrile solution of **14** also produced the cyclized bisquaternary salt **15**, but in a slower rate.

From these studies described above, it appears that the formation of cyclized bisquaternary salt can be easily achieved in acetonitrile alone or in a mixture of acetonitrile/water from the reactive nitrogen mustards HN-1 and HN-2. For less reactive HN-3, the formation of bis-quaternary salt requires a more polar environment to promote the bimolecular nucleophilic substitution.

3. EXPERIMENTAL METHODS

Melting points were determined using a Thomas-Hoover Uni-melt apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker 250 spectrometer and a Varian Unity Plus 400 Fourier Transform NMR spectrometer. Organic extracts were dried over anhydrous sodium sulfate. GC-MS spectra were obtained by Perkin Elmer AutoSystem GC/Q-Mass 910 mass spectrometer at an ionization energy of 70eV.

3.1 N,N-Bis[(2-Chloroethyl)]-N-Ethylamine Oxide Hydrochloride (6).

To a solution of sodium bicarbonate (31.5 g, 0.375 mol) in 300 mL water was added 32% peracetic acid in acetic acid (29.6 mL, 0.125 mol peracetic acid) dropwise with stirring. Carbon dioxide was generated during the the addition and final clear solution was treated with a solution of HN-3 hydrochloride (10 g, 0.04 mol), in 60 mL water, dropwise at room temperature. The resulting mixture was stirred for 1 hour and acidified with concentrated hydrochloric acid. Evaporation of the solvents under high vacuum yielded a white residue which was extracted with acetone three times. The combined acetone solution was evaporated and the light yellow oil residue was recrystallized from ethanol-ether to yield white crystalline **6** (1.34 g): mp 90-92 °C (literature[5] mp 91-92 °C). The mother liquor recovered another 0.68 g of yellow crystal, mp 88-90 °C. ^{1}H NMR (D_2O, DDS) δ 4.13 (t, 6H, $3ClCH_2$, J = 6.5 Hz), 4.28 (t, 6H, $3NCH_2$, J = 6.5 Hz); ^{13}C NMR (D_2O, DDS) 37.68, 68.66 ppm.

3.2 N,N-[Bis(2-Chloroethyl)]-N-(2-Chloroethoxy)amine (9).

Compound **6** (34 mg) was heated at 150 °C under N_2 for three minutes. The brown residue was treated with aqueous $NaHCO_3$ and extracted with 2 mL $CDCl_3$, dried, and analzed by NMR and GC/MS. ^{1}H NMR ($CDCl_3$) δ 3.78 (t, 2H, CH_2Cl, J = 6.5 Hz), 3.24 (t, 4H, $2NCH_2$, J = 6.5 Hz), 3.85 (t, 4H, $2CH_2Cl$, J = 6.5 Hz), 4.01(t, 2H, CH_2O, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) 42.93, 62.68, 62.74, 77.80 ppm; MS(EI) *m/z* 219 (M^+) (5%), 170 (100) (M^+ - CH_2Cl); GC 8.27 min.

3.3 N,N'-[Bis(2-Chloroethyl)]-N,N'-(Diethyl)piperazium Dichloride (10).

A homogeneous solution of HN-1 (**1**) (600 mg, 3.58 mmol) and 30% H_2O_2 (2 mL) in acetonitrile (3 mL) was stirred at room temperature for 16 hours and acidified with concentrated hydrochloric acid. The resulting mixture was evaporated under vacuum and the oily residue treated with EtOH. The white solid was filtered and washed with cold EtOH to give **10** (313 mg). The filtrate recovered another 250 mg of the product: mp >240 °C. The same result was obtained when acetonitrile was replaced by methanol.
A solution of HN-1 in acetonitrile at room temperature also produce **10** in quantitative yield; ^{1}H NMR (D_2O, DDS) δ 1.420 (t, 3H, CH_3, J = 7.2 Hz), 1.425 (t, 3H, CH_3, J = 7.2 Hz), 3.82 (q, 2H, CH_2, J = 7.2 Hz), 3.84 (q, 2H, CH_2, J = 7.2 Hz), 4.06-4.15 (m, 16H); ^{13}C NMR (D_2O, DDS) 10.7,38.6,56.1, 59.57, 60.12, 62.69, 63.22 ppm.

3.4 N,N'-[Bis(2-Chloroethyl)]-N,N'-(Dimethyl)piperazium Dichloride (11).

The reaction was carried out identical to the preparation of **10**, and the product **11** was obtained as a white solid: mp >220 °C; ^{1}H NMR (D_2O, DDS) δ 3.42, 3.45 (2s, 6H, $2CH_3$), 4.09-4.11 (m, 12H); ^{13}C NMR (D_2O, DDS) 37.86, 37.95, 57.49, 57.56 ppm (There is an unexpected dynamic process occurring that broadens the ^{13}C resonances of NCH_3 and NCH_2 at 48 and 72 ppm, respectively).

3.5 N,N-[Bis(2-Chloroethyl)]-N-(2-Methoxyethyl)amine (12).

To a solution of HN-3 (0.7 g, 3.4 mmol) in 18 mL MeOH was added 30% H_2O_2 (5 mL). The mixture was stirred at room temperature for 16 hours, then acidified with concentrated hydrochloric acid. Evaporation of solvents gave an oily residue which was treated with ethanol-ether to give **12** HCl (218 mg) as white crystals: mp 115-116 °C; ^{1}H NMR (D_2O, DDS) δ 3.42 (s, 3H, CH_3), 3.61 (t, 2H, NCH_2, J = 7.0 Hz), 3.79 (t, 4H, $2NCH_2$, J = 6.80 Hz), 3.86 (t, 2H, OCH_2, J = 7.0 Hz), 4.01 (t, 4H, $2ClCH_2$, J = 6.80 Hz), ^{13}C NMR (D_2O, DDS) 39.8, 55.9, 57.7, 61.2, 67.9 ppm.

3.6 N,N,N',N'-[Tetra(2-Chloroethyl)]piperazium Dichloride (13).

A solution of HN-3 (0.7 g, 3.4 mmol) and 30% H_2O_2 (2 mL) in acetonitrile (6 mL) was stirred at room temperature for 16 hours. The mixture was acidified with concentrated hydrochloric acid and the solvents were evaporated to give a foam which was treated with EtOH to yield white solid **13** (40 mg): mp >220 °C; ^{1}H NMR (D_2O, DDS) δ 4.14 (t, 8H, $4ClCH_2$, J = 6.8 Hz), 4.28 (m, 8H, $4NCH_2$), 4.30 (s, 8H); ^{13}C NMR (D_2O, DDS) 38.3, 57.14, 64.0 ppm.

3.7 N,N,N',N'-(Tetraethyl)piperazium Dichloride (15).

A solution of **14** (0.3 g) in acetonitrile (2 mL) was stirred at room temperatrue and a white solid formed in two hours. The solution was stirred for another 10 hours and the white solid was filtered, washed with cold acetonitrile, then ether to give **15** (220 mg): mp >220 °C; ^{1}H NMR (D_2O, DDS) δ 1.38 (t, 12H, $4CH_3$, J = 7.5 Hz), 3.66 (q, 8H, $4CH_2$, J = 7.5 Hz), 3.92 (s, 8H, $4CH_2$); ^{13}C NMR (D_2O, DDS) 9.22, 53.8, 57.0 ppm.

4. CONCLUSION

Tertiary amines are generally oxidized either by peracids or hydrogen peroxide to produce amine oxides that serve useful synthetic intermediates. Accordingly, the reaction of bis- or tris(2-chloroethyl)-amines (**1**~**3**) with peracetic acid yielded the corresponding N-oxides (**6**~**8**). Interestingly, hydrogen peroxide failed to convert mono-, bis- or tris(2-chloroethyl)amines to their corresponding N-oxides in methanol or acetonitrile, but rather, proceed *via* a bi-molecular nucleophilic substitution to form a cyclized bisquaternary salt. Thus, the addition of acetonitrile or methanol in the presence of a small amount of water, to nitrogen mustard rapidy forms a water soluble, nontoxic, and stable solid waste.

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